MICROFLUIDICS DEVICES AND METHODS FOR HIGH THROUGHPUT SCREENING

BACKGROUND OF THE INVENTION

This application claims priority to U.S. Provisional Applications Serial Nos. 60/204,272 and 60/204,273, each filed May 15, 2000, the disclosure of which is explicitly incorporated by reference herein.

1. Field of the Invention

This invention relates to methods and apparatus for performing microanalytic and microsynthetic analyses and procedures. In particular, the invention relates to microminiaturization of genetic, biochemical and bioanalytic processes. Specifically, the present invention provides devices and methods for the performance of miniaturized biochemical assays. These assays may be performed for a variety of purposes, including but not limited to screening of drug candidate compounds, life sciences research, and clinical and molecular diagnostics. Methods for performing any of a wide variety of such microanalytical or microsynthetic processes using the microsystems apparatus of the invention are also provided.

2. Background of the Related Art

Recent developments in a variety of investigational and research fields have created a need for improved methods and apparatus for performing analytical, particularly bioanalytical assays at microscale (*i.e.*, in volumes of less than 100 μL). In the field of pharmaceuticals, for example, an increasing number of potential drug candidates require assessment of their biological function. As an example, the field of combinatorial chemistry combines various structural sub-units with differing chemical affinities or configurations into molecules; in theory, a new molecule having potentially unique biochemical properties can be created for each permutation of the sub-units. In this way, large libraries of compounds may be synthesized from relatively small numbers of constituents, each such compound being a potential drug lead compound of usually unknown biological activity and potency.

More traditional approaches to compound library development are also yielding growing numbers of candidates, including the use of naturally-derived compounds

extracted from plants, fungi, and bacteria. In part, this is due to an increased understanding of the function of these compounds, including how they affect the metabolic pathways of the organisms which synthesize and use them; the increasing refinement in identifying and understanding compounds based on small structural and compositional differences; and improved methods for extracting and purifying these compounds.

Increased numbers of potential targets for these drug candidates are also being identified. Recent advances in biology, most notably the human genome project, have discovered many molecules whose biochemical activity is implicated in various disease states. Although these novel targets can provide exquisitely precise and specific indicia of how biological processes underlying disease can be effectively controlled and manipulated, drugs must be identified, usually by screening processes, to find compounds that can enhance, diminish, or otherwise alter these targets' ability to affect the metabolic pathways associated with disease.

The function of drug candidates, targets, and the effect of the candidates on targets is assessed in the early stages of pharmaceutical development through a process of screening that typically includes: binding of a drug candidate to a portion or domain of the target molecule; immunoassays that bind to drug candidate target domains correlated with drug efficacy; enzymatic assays, in which the inhibition of an enzymatic activity of the target by the drug candidate can be used as a sign of efficacy; protein/protein binding; and protein/DNA(RNA) binding. Additional assays involve the use of living cells and include gene expression, in which levels of transcription in response to a drug candidate are monitored, and functional assays designed to investigate both macroscopic effects, such as cell viability, as well as biochemical effects and products produced in and by the cells as a result of treatment with the drug lead compound. (Wallace & Goldman, 1997, "Bioassay Design and Implementation", in *High-Throughput Screening: The Discovery of Bioactive Substances*, J. P. Devlin, ed., Marcel Dekker, Inc.: New York, pp. 279-305).

In initial screening of compounds against targets, the number of possible screens is roughly the number of candidates multiplied by the number of targets. As a result of the growth in both the number of candidates and the number of targets, the number of assays that must be performed is growing rapidly. In addition to the increasing number of assays to be performed, it is desirable to reduce the time required to perform the assays in order to obtain results of such screenings in a timely and useful fashion.

Finally, "multiplexing" technology that allows the performance of multiple assays on one sample within a single reaction well—for example, by using readily-distinguishable signals, such as fluorescent moieties with different characteristic wavelengths—can be used to increase throughput.

In addition to drug screening assays, biological research has uncovered a vast reservoir of genetic information and diversity having little if any correlation with the function of the gene products encoded by the deciphered DNA. On the one hand, the identification of the nucleotide sequence of the human genome, coupled with bioinformatics analysis of these sequences, has identified a larger number of protein coding sequences (termed "open reading frames") that can and probably do encode functional proteins. However, since these sequences have been uncovered by simply "reading" a sequence without any information (such as the correlation of a genetic locus with a mutation associated with a disease), the function of the gene products of such a locus must be determined in order to fully understand and identify what protein target is encoded thereby and what utility drug candidates directed to such a target might have. On the other hand, human genome sequencing efforts have also identified genetic mutations (such as single nucleotide polymorphisms, or "SNPs") that may or may not be associated with human disease. In either instance, the products of this human genetic information must be assayed to determine the activity of the genes, both "wild-type" and mutant, encoded at each new genetic locus. Progress in life sciences research requires researchers to perform large numbers of assays as they investigate the structure and function of proteins coded by the growing number of identified genes in the human genome. Many of the same assays and assay formats used in drug screening may be used in other life sciences research.

Large numbers of assays must also be performed in the field of molecular diagnostics, in which individuals can now be assayed for genetic mutation associated with a disease state or the propensity to develop a disease state. For example, any particular disease or propensity for disease may be associated with several different mutations in more than one gene that can determine disease susceptibility or severity. In the monitoring of a disease state, a disease may have a "fingerprint" consisting of certain genes the expression level of which can be used diagnostically to predict the severity of the disease. Monitoring expression levels of these genes can provide an indication of the response (or lack of response) to different treatment modalities.

For these and other applications in drug discovery, life sciences research, and molecular and clinical diagnostics there exists a need for systems and assay methods that can perform very many assays in a highly-parallel fashion at low cost. A central strategy has been the miniaturization of existing assays or development of new assays that work with very small volumes of drug compound and reagents. Miniaturization has been accompanied by the development of more sensitive detection schemes, including both better detectors for conventional signals (*e.g.,* colorimetric absorption, fluorescence, and chemiluminescence) as well as new chemistries or assay formats (*e.g.,* imaging, optical scanning, and confocal microscopy).

Miniaturization can also confer performance advantages. At short length scales, diffusionally-limited mixing is rapid and can be exploited to create sensitive assays (Brody *et al.,* 1996, *Biophysical J.* 71: 3430-3431). Because fluid flow in miniaturized pressure-driven systems is laminar, rather than turbulent, processes such as washing and fluid replacement are well-controlled. Microfabricated systems also enable assays that rely on a large surface area to volume ratio such as those that require binding to a surface and a variety of chromatographic approaches.

The development of fluid-handling and processing for miniaturized assays has primarily involved the scaling down of conventional methods. The vast majority of initial drug screens have been performed conventionally in 96-well microtiter plates with operating volumes of less than 0.1-0.5mL. The wells of these plates serve as "test tubes" for reactions as well as optical cuvettes for detection. Fluids are typically delivered to these plates using automated pipetting stations or external tubing and pumps; automation is also required for handling of plates and delivery to sub-systems such as plate washers (used in solid phase assays, for example).

Miniaturization has led to the creation of 384-well and 1536-well microtiter plates for total reaction volumes of between 0.015 and 0.1mL. However, a number of problems arise when miniaturizing standard plate technology. First, because the total volumes are smaller and the plates are open to the environment, evaporation of fluid during the course of an assay can compromise results. Another drawback of open plates is the existence of a fluid meniscus in the well. Meniscuses of varying configurations (due, *for example* to imperfections in the plate or differences in contact angle and surface tension) can distort the optical signals used to interrogate the samples. As the strength of the optical signals decreases with decreasing assay volume, correction for background distortions becomes more difficult. Finally, optical scanning systems for

high-density plates are often complex and expensive. Methods that minimize evaporation, provide a more uniform optical pathway, and provide simpler detection schemes are desirable.

Highly accurate pipetting technologies have been developed to deliver fluids in precisely metered quantities to these plates. Most of these fluid-delivery methods for low volumes (below a few microliters) rely on expensive piezoelectric pipetting heads that are complex and difficult to combine or "gang" into large numbers of independent pipettors so that many wells may be addressed independently. As a result, fluid delivery is either completely or partially serial (*i.e.*, a single micropipettor, or a small number of parallel delivery systems used repeatedly to address the entire plate). Serial pipetting defeats the aim of parallelism by increasing the amount of time required to address the plate. Methods that reduce the number and precision of fluid transfer steps are therefore needed.

Integration of microdevices with existent laboratory infrastructure is also desirable and has been poorly addressed in the art. This integration is one of both scale and format. Regarding scale, fluids must be transferred to devices from the external world, where the volumes in which they are handled are typically one or more orders of magnitude greater than the volumes required by the microdevice. It is desirable that this transition be done in a way that does not introduce excessively complex processes or machinery and which does not create excessive errors, such as in the volume of fluid transferred. Regarding format, it is desirable that microdevices have a similar physical aspect to macroscale devices already used in laboratories, especially in regard to the manner in which fluids are added to or removed from the devices. Microdevices that can be loaded with fluids using standard methods, such as pipettors, will be more easily and widely used in a variety of settings.

Fluid processing in microtiter plates is also difficult. The small dimensions of the wells, while enhancing diffusional mixing, suppress turbulence and make difficult mixing on length scales between a few tens of microns and a few millimeters. For similar reasons, washing, an important step in many assays can be problematic. Methods that reduce both the number of manipulations of fluids on the plate as well as manipulations of the plate itself (such as passing the plate to and from washing stations) can reduce cost while improving assay quality through suppression of contamination, carry-over, and fluid loss.

Thus, there is a need in the art for improved micromanipulation apparatus and methods for performing bioanalytic assays more rapidly and economically using less biological sample material and which may be easily interfaced with existing laboratory instrumentation. Relevant to this need in the art, some of the present inventors have developed a microsystem platform and a micromanipulation device to manipulate said platform by rotation, thereby utilizing the centripetal forces resulting from rotation of the platform to motivate fluid movement through microchannels embedded in the microplatform, as disclosed in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000, and co-owned and co-pending patent applications U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; 08/910,726, filed August 12, 1997; 08/995,056, filed December 19, 1997; and 09/315,114, filed May 19, 1999, the disclosures of each of which are explicitly incorporated by reference herein.

SUMMARY OF THE INVENTION

This invention provides microsystems platforms as disclosed in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000, and co-owned and co-pending patent applications U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; 08/910,726, filed August 12, 1997; 08/995,056, filed December 19, 1997; and 09/315,114, filed May 19, 1999, the disclosures of each of which are explicitly incorporated by reference herein.

The invention provides apparatus and methods for performing microscale processes on a microplatform, whereby fluid is moved on the platform in defined channels motivated by centripetal force arising from rotation of the platform. The first element of the apparatus of the invention is a microplatform that is a rotatable structure, most preferably a disk, the disk comprising fluid (sample) inlet ports, fluidic microchannels, reagent reservoirs, collection chambers, detection chambers and sample outlet ports, generically termed "microfluidic structures." The disk is rotated at speeds from about 1 to about 30,000 rpm for generating centripetal acceleration that enables fluid movement through the microfluidic structures of the platform. The disks of the invention also preferably comprise air outlet ports and air displacement channels. The air outlet ports and in particular the air displacement ports provide a means for fluids to displace air, thus ensuring uninhibited movement of fluids on the disk. Specific sites on

the disk also preferably comprise elements that allow fluids to be analyzed, as well as detectors for each of these effectors.

The discs of this invention have several advantages over those that exist in the centrifugal analyzer art. Foremost is the fact that flow is laminar due to the small dimensions of the fluid channels; this allows for better control of processes such as mixing and washing. Secondly, the small dimensions conferred by microfabrication enable the use of "passive" valving, dependent upon capillary forces, over much wider ranges of rotational velocities and with greater reliability than in more macroscopic systems. To this are added the already described advantages of miniaturization.

The second element of the invention is a micromanipulation device that is a disk player/ reader device that controls the function of the disk. This device comprises mechanisms and motors that enable the disk to be loaded and rotated. In addition, the device provides means for a user to operate the microsystems in the disk and access and analyze data, preferably using a keypad and computer display. The micromanipulation device also advantageous provides means for actuation of on-disc elements, such as active valves; the application and control of heat to the disc for purposes of chemical or biological incubation; and means for adding fluids to and removing fluids from the discs. The micromanipulation devices of this invention are more particularly described in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000, and co-owned and co-pending patent applications U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; 08/910,726, filed August 12, 1997; 08/995,056, filed December 19, 1997; and 09/315,114, filed May 19, 1999, the disclosures of each of which are explicitly incorporated by reference herein.

The invention specifically provides microsystems platforms comprising microfluidics components contained in one or a multiplicity of platform layers that are fluidly connected to permit transfer, mixing and assay performance on the sealed surface of the platform. The platforms preferably comprise reagent reservoirs containing a sufficient volume, preferably from about 1nL to about 1mL, of a reagent solution for a multiplicity of individual assays. The reagent reservoirs are fluidly connected by microchannels to one or more preferably a multiplicity of collection, and more preferably detection, chambers, and the microfluidics components arranged so that a specific volume of the reagent solution is delivered to each collection chamber. More preferably, said reagent reservoirs are fluidly connected to mixing structures, most preferably a mixing microchannel that is also fluidly connected to a sample reservoir, so

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that one or a plurality of reagents are mixed with sample and the resulting mixture delivered into the detection chamber. In preferred embodiments, the platform comprises a multiplicity of sample reservoirs and mixing structures fluidly connected with a multiplicity of detection chambers.

In the use of the platforms of the invention, fluids (including samples and reagents) are added to the platform when the platform is at rest. Thereafter, rotation of the platform on a simple motor motivates fluid movement through microchannels for various processing steps. In preferred embodiments, the platforms of the invention permit the use of a detector, most preferably an optical detector, for detecting the products of the assay, whereby the collection chambers comprise optical cuvettes, preferably at the outer edge of the platform, most preferably wherein the platform is scanned past a fixed detector through the action of the rotary motor. Because the platforms of the invention are most preferably constructed using microfabrication techniques as described more fully below, the volumes of fluids used may be made arbitrarily small as long as the detectors used have sufficient sensitivity.

The present invention solves problems in the current art through the use of a microfluidic disc in which centripetal acceleration is used to move fluids. It is an advantage of the microfluidics platforms of the present invention that the fluid-containing components are constructed to contain a small volume, thus reducing reagent costs, reaction times and the amount of biological material required to perform an assay. It is also an advantage that the fluid-containing components are sealed, thus eliminating experimental error due to differential evaporation of different fluids and the resulting changes in reagent concentration. Because the microfluidic devices of the invention are completely enclosed, both evaporation and optical distortion are reduced to negligible levels. The platforms of the invention also advantageously permit "passive" mixing and valving, *i.e.*, mixing and valving are performed as a consequence of the structural arrangements of the components on the platforms (such as shape, length, position on the platform surface relative to the axis of rotation, and surface properties of the interior surfaces of the components, such as wettability as discussed below), and the dynamics of platform rotation (speed, acceleration, direction and change-of-direction), and permit control of assay timing and reagent delivery.

The disclosed invention is a microfluidic disc comprising metering structures and a microfluidic network that is used to distribute aliquots of reagent to each of a multiplicity of mixing structures, each mixing structure being fluidly connected to one

of a multiplicity of sample reservoirs, thereby permitting parallel processing and mixing of the samples with one or more common reagents. The fluidic network, defined as the overall pattern of channels, reservoirs, microvalves, and air vents, may be planar or three-dimensional, depending upon the application under consideration. The use of such metering and distribution reduces the need for automated reagent distribution mechanisms, reduces the amount of time required for reagent dispensing (that can be performed in parallel with distribution of reagent to a multiplicity of reaction chambers), and permits delivery of small (nL-to-µL) volumes without using externally-applied electromotive means.

The assembly of a multiplicity of collection chambers on the platforms of the invention also permits simplified detectors to be used, whereby each individual collection/detection chamber can be scanned using mechanisms well-developed in the art for use with, *for example*, CD-ROM technology. Finally, the platforms of the invention are advantageously provided with sample and reagent entry ports for filling with samples and reagents, respectively, that can be adapted to liquid delivery means known in the art (such as micropipettors).

The platforms of the invention reduce the demands on automation in at least three ways. First, the need for precise metering of delivered fluids is relaxed through the use of on-disc metering structures, as described more fully in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000, and co-owned and co-pending patent applications U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; 08/910,726, filed August 12, 1997; 08/995,056, filed December 19, 1997; and 09/315,114, filed May 19, 1999, the disclosures of each of which are explicitly incorporated by reference herein. By loading imprecise volumes, slightly in excess of those needed for the assay, and allowing the rotation of the disc and use of appropriate microfluidic structures to meter the fluids, much simpler (and less expensive) fluid delivery technology may be employed than is the conventionally required for high-density microtitre plate assays.

Second, the total number of fluid "delivery" events on the microfluidic platform is reduced relative to microtiter plates. By using microfluidic structures that sub-divide and aliquot common reagents (such as reagent solutions, buffers, and enzyme substrates) used in all assays performed on the platform, the number of manual or automated pipetting steps are reduced by at least half (depending on the complexity of

the assay). Examples of these structures have been disclosed in co-owned U.S. Patent 6,063,589, issued May 16, 2000, and incorporated by reference herein; and are disclosed below.

Certain preferred embodiments of the apparatus of the invention are described in greater detail in the following sections of this application and in the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts an exploded, oblique view of a microsystems platform of the invention.

Figure 2a depicts a plan view of the upper face of one component of the microsystems platform shown in exploded, oblique view in Figure 1, the reservoir layer.

Figure 2b is a detail of one component of the upper face of the reservoir layer illustrated in Figure 2a.

Figure 3 is a plan view of another component of the microsystems platform of Figure 1, the upper sealing film.

Figure 4a depicts a plan view of the lower face of the reservoir layer.

Figure 4b is a detail of the lower face of the reservoir layer showing the second reagent addition reservoir and first reagent overflow reservoir.

Figure 4c is a detail of the lower face of the reservoir layer showing the first reagent addition reservoir and second reagent overflow reservoir.

Figure 4d is a detail of the lower face of the reservoir layer showing one series of sample and reagent reservoirs, channels, and a detection cuvette for a single microdevice that performs a single measurement.

Figure 5a depicts a plan view of another component of the microsystems platform of Figure 1, the microfluidic layer.

Figure 5b is a detail of one segment of the microfluidic layer of Figure 5 comprising the microfluidic channels of a single microfluidic assay structure.

Figure 5c is a detail of the microfluidic layer of Figure 5 illustrating the overflow valve and channels for the first reagent.

Figure 5d is a detail of the microfluidic layer of Figure 5 illustrating the overflow valve and channels for the second reagent.

Figure 6 is a segment of the assembled reservoir and microfluidic layers comprising the microsystems platform of Figure 1.

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Figures 7a through 7g illustrate the sequence of fluid motions as sample, reagent 1, and reagent 2 are distributed to the reservoirs of the device of Figure 1.

Figures 8a through 8l illustrate the sequence of fluid motions into a single microfluidic assay structure of the device of Figure 1.

Figure 9 illustrates a first alternative construction of the microsystems platform in which the sample volume is metered by the construction of the device.

Figure 10 illustrates a second alternative construction of the microsystems platform in which two common reagents are isolated through the introduction of a bubble.

Figure 11 illustrates a third alternative construction of the microsystems platform in which a series of mixtures of two reagents are delivered to the assay structures of the device.

Figure 12 shows a device developed for the performance of 24 parallel assays.

Figure 13 shows a dose-response curve illustrating enzymatic activity as a function of inhibitor concentration for enzymatic inhibition assays performed with the devices of the invention, as disclosed in Example 1 and illustrated in Figure 12.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

This invention provides a microplatform and a micromanipulation device as disclosed in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000, and co-owned and co-pending patent applications U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; 08/910,726, filed August 12, 1997; 08/995,056, filed December 19, 1997; 09/315,114, filed May 19, 1999, the disclosures of each of which are explicitly incorporated by reference herein, adapted for performing microanalytical and microsynthetic assays of biological samples.

For the purposes of this invention, the term "sample" will be understood to encompass any fluid, solution or mixture, either isolated or detected as a constituent of a more complex mixture, or synthesized from precursor species. In particular, the term "sample" will be understood to encompass any biological species of interest. The term "biological sample" or "biological fluid sample" will be understood to mean any biologically-derived sample, including but not limited to blood, plasma, serum, lymph, saliva, tears, cerebrospinal fluid, urine, sweat, plant and vegetable extracts, semen, and ascites fluid.

For the purposes of this invention, the term "a centripetally motivated fluid micromanipulation apparatus" is intended to include analytical centrifuges and rotors, microscale centrifugal separation apparatuses, and most particularly the microsystems platforms and disk handling apparatuses as described in co-owned U.S. Patent No.

5   6,063,589, issued May 16, 2000, and co-owned and co-pending patent applications U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; 08/910,726, filed August 12, 1997; 08/995,056, filed December 19, 1997; 09/315,114, filed May 19, 1999, the disclosures of each of which are explicitly incorporated by reference herein.

10   For the purposes of this invention, the term "microsystems platform" is intended to include centripetally-motivated microfluidics arrays as described in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000, and co-owned and co-pending patent applications U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; 08/910,726, filed August 12, 1997; 08/995,056, filed December

15   19, 1997; 09/315,114, filed May 19, 1999, the disclosures of each of which are explicitly incorporated by reference herein.

For the purposes of this invention, the terms "capillary", "microcapillary" and "microchannel" will be understood to be interchangeable and to be constructed of either wetting or non-wetting materials where appropriate.

20   For the purposes of this invention, the term "reservoir," "assay chamber," "fluid holding chamber," "collection chamber" and "detection chamber" will be understood to mean a defined volume on a microsystems platform of the invention comprising a fluid.

For the purposes of this invention, the terms "entry port" and "fluid input port" will be understood to mean an opening on a microsystems platform of the invention

25   comprising a means for applying a fluid to the platform.

For the purposes of this invention, the terms "exit port" and "fluid outlet port" will be understood to mean a defined volume on a microsystems platform of the invention comprising a means for removing a fluid from the platform.

For the purposes of this invention, the term "capillary junction" will be

30   understood to mean a region in a capillary or other flow path where surface or capillary forces are exploited to retard or promote fluid flow. A capillary junction is provided as a pocket, depression or chamber in a hydrophilic substrate that has a greater depth (vertically within the platform layer) and/ or a greater width (horizontally within the platform layer) that the fluidics component (such as a microchannel) to which it is

fluidly connected. For liquids having a contact angle less than 90° (such as aqueous solutions on platforms made with most plastics, glass and silica), flow is impeded as the channel cross-section increases at the interface of the capillary junction. The force hindering flow is produced by capillary pressure, that is inversely proportional to the cross sectional dimensions of the channel and directly proportional to the surface tension of the liquid, multiplied by the cosine of the contact angle of the fluid in contact with the material comprising the channel. The factors relating to capillarity in microchannels according to this invention have been discussed in co-owned U.S. Patent No. 6,063,589, issued May 12, 2000 and in co-owned and co-pending U.S. patent application, Serial No. 08/910,726, filed August 12, 1997, incorporated by reference in its entirety herein.

Capillary junctions can be constructed in at least three ways. In one embodiment, a capillary junction is formed at the junction of two components wherein one or both of the lateral dimensions of one component is larger than the lateral dimension(s) of the other component. As an example, in microfluidics components made from "wetting" or "wettable" materials, such a junction occurs at an enlargement of a capillary as described in co-owned and co-pending U.S. Serial Nos. U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; and 08/910,726, filed August 12, 1997. Fluid flow through capillaries is inhibited at such junctions. At junctions of components made from non-wetting or non-wettable materials, on the other hand, a constriction in the fluid path, such as the exit from a chamber or reservoir into a capillary, produces a capillary junction that inhibits flow. In general, it will be understood that capillary junctions are formed when the dimensions of the components change from a small diameter (such as a capillary) to a larger diameter (such as a chamber) in wetting systems, in contrast to non-wettable systems, where capillary junctions form when the dimensions of the components change from a larger diameter (such as a chamber) to a small diameter (such as a capillary).

A second embodiment of a capillary junction is formed using a component having differential surface treatment of a capillary or flow-path. For example, a channel that is hydrophilic (that is, wettable) may be treated to have discrete regions of hydrophobicity (that is, non-wettable). A fluid flowing through such a channel will do so through the hydrophilic areas, while flow will be impeded as the fluid-vapor meniscus impinges upon the hydrophobic zone.

The third embodiment of a capillary junction according to the invention is provided for components having changes in both lateral dimension and surface properties. An example of such a junction is a microchannel opening into a hydrophobic component (microchannel or reservoir) having a larger lateral dimension. Those of ordinary skill will appreciate how capillary junctions according to the invention can be created at the juncture of components having different sizes in their lateral dimensions, different hydrophilic properties, or both.

For the purposes of this invention, the term "capillary action" will be understood to mean fluid flow in the absence of rotational motion or centripetal force applied to a fluid on a rotor or platform of the invention and is due to a partially or completely wettable surface.

For the purposes of this invention, the term "capillary microvalve" will be understood to mean a capillary microchannel comprising a capillary junction whereby fluid flow is impeded and can be motivated by the application of pressure on a fluid, typically by centripetal force created by rotation of the rotor or platform of the invention. Capillary microvalves will be understood to comprise capillary junctions that can be overcome by increasing the hydrodynamic pressure on the fluid at the junction, most preferably by increasing the rotational speed of the platform.

For the purposes of this invention, the term "in fluid communication" or "fluidly connected" is intended to define components that are operably interconnected to allow fluid flow between components.

For the purposes of this invention, the term "air displacement channels" will be understood to include ports in the surface of the platform that are contiguous with the components (such as microchannels, chambers and reservoirs) on the platform, and that comprise vents and microchannels that permit displacement of air from components of the platforms and rotors by fluid movement.

The microplatforms of the invention (preferably and hereinafter collectively referred to as "disks"; for the purposes of this invention, the terms "microplatform", "microsystems platform" and "disk" are considered to be interchangeable) are provided to comprise one or a multiplicity of microsynthetic or microanalytic systems (termed "microfluidics structures" herein). Such microfluidics structures in turn comprise combinations of related components as described in further detail herein that are operably interconnected to allow fluid flow between components upon rotation of the disk. These components can be microfabricated as described below either integral to

the disk or as modules attached to, placed upon, in contact with or embedded in the disk. For the purposes of this invention, the term "microfabricated" refers to processes that allow production of these structures on the sub-millimeter scale. These processes include but are not restricted to molding, photolithography, etching, stamping and other

5   means that are familiar to those skilled in the art.

The invention also comprises a micromanipulation device for manipulating the disks of the invention, wherein the disk is rotated within the device to provide centripetal force to effect fluid flow on the disk. Accordingly, the device provides means for rotating the disk at a controlled rotational velocity, for stopping and starting

10   disk rotation, and advantageously for changing the direction of rotation of the disk. Both electromechanical means and control means, as further described herein, are provided as components of the devices of the invention. User interface means (such as a keypad and a display) are also provided, as further described in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000, and co-owned and co-pending patent applications

15   U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; 08/910,726, filed August 12, 1997; 08/995,056, filed December 19, 1997; 09/315,114, filed May 19, 1999, the disclosures of each of which are explicitly incorporated by reference herein.

The invention provides a combination of specifically-adapted microplatforms

20   that are rotatable, analytic/synthetic microvolume assay platforms, and a micromanipulation device for manipulating the platform to achieve fluid movement on the platform arising from centripetal force on the platform as result of rotation. The platform of the invention is preferably and advantageously a circular disk; however, any platform capable of being rotated to impart centripetal for a fluid on the platform is

25   intended to fall within the scope of the invention. The micromanipulation devices of the invention are more fully described in co-owned and co-pending U.S. Serial Nos. U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; 08/910,726, filed August 12, 1997; 08/995,056, filed December 19, 1997; and 09/315,114, filed May 19, 1999, the disclosures of each of which are explicitly

30   incorporated by reference herein.

Fluid (including reagents, samples and other liquid components) movement is controlled by centripetal acceleration due to rotation of the platform. The magnitude of centripetal acceleration required for fluid to flow at a rate and under a pressure appropriate for a particular microfluidics structure on the microsystems platform is

determined by factors including but not limited to the effective radius of the platform, the interior diameter of microchannels, the position angle of the microchannels on the platform with respect to the direction of rotation, and the speed of rotation of the platform. In certain embodiments of the methods of the invention an unmetered amount of a fluid (either a sample or reagent solution) is applied to the platform and a metered amount is transferred from a fluid reservoir to a microchannel, as described in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000, and co-owned and co-pending patent applications U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; 08/910,726, filed August 12, 1997; 08/995,056, filed December 19, 1997; 09/315,114, filed May 19, 1999, the disclosures of each of which are explicitly incorporated by reference herein. In preferred embodiments, the metered amount of the fluid sample provided on an inventive platform is from about 1nL to about 500µL. In these embodiments, metering manifolds comprising one or a multiplicity of metering capillaries are provided to distribute the fluid to a plurality of components of the microfluidics structure.

The components of the platforms of the invention are in fluidic contract with one another. In preferred embodiments, fluidic contact is provided by microchannels comprising the surface of the platforms of the invention. Microchannel sizes are optimally determined by specific applications and by the amount of and delivery rates of fluids required for each particular embodiment of the platforms and methods of the invention. Microchannel sizes can range from 0.1 µm to a value close to the thickness of the disk (e.g., about 1mm); in preferred embodiments, the interior dimension of the microchannel is from 0.5µm to about 500µm. Microchannel and reservoir shapes can be trapezoid, circular or other geometric shapes as required. Microchannels preferably are embedded in a microsystem platform having a thickness of about 0.1 to 25mm, wherein the cross-sectional dimension of the microchannels across the thickness dimension of the platform is less than 1mm, and can be from 1 to 90 percent of said cross-sectional dimension of the platform. Sample reservoirs, reagent reservoirs, reaction chambers, collection chambers, detections chambers and sample inlet and outlet ports preferably are embedded in a microsystem platform having a thickness of about 0.1 to 25mm, wherein the cross-sectional dimension of the microchannels across the thickness dimension of the platform is from 1 to 75 percent of said cross-sectional dimension of the platform. In preferred embodiments, delivery of fluids through such channels is achieved by the coincident rotation of the platform for a time and at a

rotational velocity sufficient to motivate fluid movement between the desired components.

The flow rate through a microchannel of the invention is inversely proportional to the length of the longitudinal extent or path of the microchannel and the viscosity of the fluid and directly proportional to the product of the square of the hydraulic diameter of the microchannel, the square of the rotational speed of the platform, the average distance of the fluid in the channels from the center of the disk and the radial extent of the fluid subject to the centripetal force. Since the hydraulic diameter of a channel is proportional to the ratio of the cross-sectional area to cross-sectional perimeter of a channel, one can judiciously vary the depth and width of a channel to affect fluid flow (*see* Duffy *et al.*, 1998, *Anal. Chem.* 71: 4669-4678 and co-owned and co-pending patent applications U.S. Serial Nos. 08/761,063, filed December 5, 1996 and 08/768,990, filed December 18, 1996, incorporated by reference).

For example, fluids of higher densities flow more rapidly than those of lower densities given the same geometric and rotational parameters. Similarly, fluids of lower viscosity flow more rapidly than fluids of higher viscosity given the same geometric and rotational parameters. If a microfluidics structure is displaced along the radial direction, thereby changing the average distance of the fluid from the center of the disc but maintaining all other parameters, the flow rate is affected: greater distances from the center result in greater flow rates. An increase or a decrease in the radial extent of the fluid also leads to an increase or decrease in the flow rate. These depencies are all linear. Variation in the hydraulic diameter results in a quartic dependence of flow rate on hydraulic diameter (or quadratic dependence of fluid flow velocity on hydraulic diameter), with larger flow rates corresponding to larger diameters. Finally, an increase in the rotational rate results in a quadratic increase in the flow rate or fluid flow velocity.

Input and output (entry and exit) ports are components of the microplatforms of the invention that are used for the introduction or removal of fluid components. Entry ports are provided to allow samples and reagents to be placed on or injected onto the disk; these types of ports are generally located towards the center of the disk. Exit ports are also provided to allow products to be removed from the disk. Port shape and design vary according specific applications. For example, sample input ports are designed, *inter alia*, to allow capillary action to efficiently draw the sample into the disk. In addition, ports can be configured to enable automated sample/reagent loading or

product removal. Entry and exit ports are most advantageously provided in arrays, whereby multiple samples are applied to the disk or to effect product removal from the microplatform.

In some embodiments of the platforms of the invention, the inlet and outlet ports are adapted to the use of manual pipettors and other means of delivering fluids to the reservoirs of the platform. In alternative, advantageous embodiments, the platform is adapted to the use of automated fluid loading devices. One example of such an automated device is a single pipette head located on a robotic arm that moves in a direction radially along the surface of the platform. In this embodiment, the platform could be indexed upon the spindle of the rotary motor in the azimuthal direction beneath the pipette head, which would travel in the radial direction to address the appropriate reservoir.

Another embodiment is a pipettor head adapted to address multiple reservoirs, either a subset of or all of the reservoirs on the platform surface. For embodiments where the pipettor head addresses a subset of the reservoirs, a single head may for example be composed of a linear array of pipette heads. For example, the entry ports of Figure 1 might be addressed by indexing such a linear head in the direction transverse to the pipette tips. In other embodiments, pipette heads may be used which can simultaneously address all entry ports (for example, a 96-tip head). In these embodiments, there may be a distinction between sample entry ports—needed for the delivery of many samples—and reagent entry ports, through which larger volumes or reagent are delivered for use in reactions with all samples. A pipetting device that can simultaneously address all sample entry ports as well as reagent ports might consist of a standard multipipettor with a few added, large-volume delivery tips.

Also included in air handling systems on the disk are air displacement channels, whereby the movement of fluids displaces air through channels that connect to the fluid-containing microchannels retrograde to the direction of movement of the fluid, thereby providing a positive pressure to further motivate movement of the fluid.

Platforms of the invention such as disks and the microfluidics components comprising such platforms are advantageously provided having a variety of composition and surface coatings appropriate for particular applications. Platform composition will be a function of structural requirements, manufacturing processes, and reagent compatibility/chemical resistance properties. Specifically, platforms are provided that are made from inorganic crystalline or amorphous materials, *e.g.* silicon, silica, quartz,

inert metals, or from organic materials such as plastics, for example, poly(methyl methacrylate) (PMMA), acetonitrile-butadiene-styrene (ABS), polycarbonate, polyethylene, polystyrene, polyolefins, polypropylene and metallocene. These may be used with unmodified or modified surfaces as described below. The platforms may also be made from thermoset materials such as polyurethane and poly(dimethyl siloxane) (PDMS). Also provided by the invention are platforms made of composites or combinations of these materials; *for example*, platforms manufactures of a plastic material having embedded therein an optically transparent glass surface comprising the detection chamber of the platform. Alternately, platforms composed of layers made from different materials may be made. The surface properties of these materials may be modified for specific applications, as disclosed in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000, and co-owned and co-pending patent applications U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; 08/910,726, filed August 12, 1997; 08/995,056, filed December 19, 1997; and 09/315,114, filed May 19, 1999, the disclosures of each of which are explicitly incorporated by reference herein.

Preferably, the disk incorporates microfabricated mechanical, optical, and fluidic control components on platforms made from, *for example*, plastic, silica, quartz, metal or ceramic. These structures are constructed on a sub-millimeter scale by molding, photolithography, etching, stamping or other appropriate means, as described in more detail below. It will also be recognized that platforms comprising a multiplicity of the microfluidic structures are also encompassed by the invention, wherein individual combinations of microfluidics and reservoirs, or such reservoirs shared in common, are provided fluidly connected thereto. An example of such a platform is shown in Figure 1.

Platform Manufacture and Assembly

Referring now to the Figures for a more thorough description of the invention, Figure 1 shows an exploded view of an example of a disc appropriate for large numbers of similar or identical microfluidics structures for performing, *inter alia*, liquid-phase assays. The disc shown here enables the performance of 96 parallel assays of the same form. The assays have the general form: mix first fluid A with second fluid B, and then mix the combined fluids (A+B) with third fluid C. In practice, the fluid A may actually be a number of fluids, A_1, A_2,..A_i,...A_n, where n is

the total number of assays to be performed on independent fluids. Furthermore, the disc is designed for loading fluids thereupon through ports constructed in the platform. For example, one fluid, A, herein termed "sample", is loaded through 96 independent entry ports. The second fluid, B, is loaded in a volume somewhat greater than 96 times the volume required for each assay into a single entry port. The third fluid, C, is similarly loaded into a single entry port. The volumes of each of the reservoirs containing fluids A, B and C, or the amount of fluid A, B or C loaded onto the disk, can be different, depending on the requirements of the assay. The disc is configured so that rotation of the disc after loading, under a prescribed rotational profile, effects the following fluid motions: Delivery of individual samples A_i to assay structures within the disc; delivery of metered aliquots of fluid B to individual assay structures within the disc; delivery of metered aliquots of fluid C to individual assay structures within the disc; "isolation" of individual assay structure volumes of fluids B and C through the use of overflow reservoirs which take excess fluid and introduce air bubbles between individual assay structures; and the performance of the assay as described above, wherein the discs effect two fluid mixing steps. The number of fluids and sequence of mixing steps can be arbitrary, *e.g.*, $(A_i + B) + (B+C)$. The total number of fluids can also be arbitrary, and the three-dimensional nature of the microfluidic network allows the "crossing over" of numerous channels. Features of this device include: a) "samples" that must be loaded in large numbers can be loaded into a standard format accessible to laboratory robotics or standard automated pipetting systems; b) common reagents need to be loaded only once each, and without high precision. These features integrate the device into existing laboratory infrastructure while providing the advantages of reduced operating time for fluid transfers, enclosed assays for reduction of evaporation and contamination, and the removal of liquid-air meniscus for detection.

This disc illustrates that identical assays may be made by repeating assay structures around the disc at a given radius as well as modifying the structures for placement at different radial positions. In this way, it is possible to fully cover the surface of the disc with microfluidics structures for performing assays. The maximum number of assays that may be performed will depend upon the volume of fluid that cay be manipulated reproducibly, *i.e.*, the minimum reproducible dimensions with which the disc may be fabricated, and the amount of hydrodynamic pressure required to drive small volumes of fluid through microchannels at convenient rotational rates. Taking

these considerations into account, it is estimated that greater than 10,000 assays having volumes of 1-5nL can be created in a circular platform having a 6cm radius.

In Figure 1, platform **100** is composed of at least 3 component layers. A reservoir layer **201** having features on the lower face, or both the upper and lower

5   faces, is used. In cases where there are fluidic channels on the upper surface, a sealing film **301** is used to enclose those channels. The "lower" surface of the reservoir layer **201** is bonded to a microfluidics layer **500**. The upper surface of the reservoir layer contains the fluid entry ports; it may also contain one or more distribution manifolds as described herein for distributing one or more common fluids. The bottom face of the

10   reservoir layer, when mated with the microfluidic layer described below, forms a complete network of enclosed channels and reservoirs through which fluids flows under the impetus of centripetal force created by rotation of the platform about a central axis. Fluid flow permits mixing of various component fluids in the assay and movement of the fluids from sample and reagent chambers through mixing structures

15   and into assay reaction chambers. In addition, fluid flow can be effectuated to include incubation and wash steps, using structures disclosed in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000 and incorporated by reference herein. Fluid flow rates range from about 1nL/s to about 1000μL/s at rotational speeds of from about 4-30,000rpm. "Passive" or capillary valves are preferably used to control fluid flow in

20   the platform as described in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000, and co-owned and co-pending patent applications U.S. Serial Nos. 08/761,063, filed December 5, 1996; 08/768,990, filed December 18, 1996; 08/910,726, filed August 12, 1997; 08/995,056, filed December 19, 1997; 09/315,114, filed May 19, 1999, the disclosures of each of which are explicitly incorporated by reference herein.

25   In the operation of the platforms of the invention, competition between rotationally-induced hydrostatic pressure and the capillary pressure exerted in small channels and orifices are exploited to provide a rotation-depending gating or valving system. After fluids are deposited in detection chambers positioned towards the outer edge of the platform, an optical signal is detected.

30   Platform **100** is preferably provided in the shape of a disc, a circular planar platform having a diameter of from about 10 mm to about 50mm and a thickness of from about 0.1mm to about 25mm. Each layer comprising the platform preferably has a diameter that is substantially the same as the other layers, although in some

embodiments the diameters of the different layers are not required to completely match. Each layer has a thickness ranging from about 0.1mm to about 25mm, said thickness depending in part on the volumetric capacity of the microfluidics components contained therein.

Reservoir Layer

The structure of top surface of the reservoir layer **201** is shown in Figure 2a.

Reservoir layer **201** is preferably provided in the shape of a disc, a circular planar platform having a diameter of from about 10mm to about 50mm and a thickness of from about 0.1mm to about 25mm. The layer preferably comprises a center hole **202** for mounting on a spindle, having a diameter of from about 1mm to about 20mm. Center hole **202** can be replaced by an extruded fitting for connection to a spindle, or may be absent entirely, in which case registry and connection to the spindle is accomplished using the attached microfluidic layer or another portion of the surface of the platform.

Figure 2a illustrates a variety of structures necessary for device function. These include "sample" or fluid entry ports that are comprised of a through hole **204** communicating between the two faces of the disc and in some embodiments a conical or cup-like depression **203.** The depression aids in the placement of pipette tips when the device is used manually. These entry holes are typically arrayed in a rectangular pattern with a spacing to permit an automated pipetting device such as an 8-tip linear or 96-tip rectangular pipetting head to be used. For such devices the spacing of entry ports is typically 9mm or 4.5mm, and the arrays are typically 8×12 or 16×24 elements in size. These ports may also be placed in the pattern of a 1536-well plate, which has a spacing of 2.25mm and consists of 32×48 elements. They may also be placed in an arbitrary pattern for manual use or use with custom devices. The upper surface also contains entry ports for the addition of the two common reagents, comprised respectively of **214+215** and **216+217.** These ports have dimensions adapted to automated loading devices such as micropipettors, for example, a standard 200μL plastic pipette tip of tip diameter 1.5mm; micropipette tips of diameter 1mm; piezoelectric or ceramic drop delivery systems; and inkjet-based fluid delivery systems. For non-contact delivery systems such as piezoelectric or inkjet delivery, the dimensions of the ports must be a few times greater than the size of the droplets, *e.g.,* 0.2mm for a 1nL drop.

Corresponding to these latter entry ports are air ports **205** and **206** that allow air to escape from the common reagent reservoirs **401** and **402** (discussed below). The disc is able to distribute three fluids to an arbitrary port only by having a three-dimensional construction: fluid paths must "cross over" one another. As a result, some of the fluid

5 motion occurs on the upper face of **201**. Reagent aliquotting manifold **210** is such a distribution channel; its connection to bulk reagent reservoir **402** is *via* through hole **207** and exit channel **208**. Along the manifold **210** are vias **211** which penetrate from one side of the disc to the other, allowing fluids to be distributed from the top to the bottom of the disc. The manifold **210** terminates in a via **218** which communicates with an

10 overflow reservoir **403** discussed below. Also visible on the upper surface are air-ports **212** and **213** whose function will be discussed further below.

Figure 2b is a detail of the fluid entry to the manifold. The through-hole **207** connects the upper surface of the disc within the channel **208** to the reservoir **402** on the lower surface of the disc.

15 Figure 3 shows the sealing film **301**. The sealing film is typically of a thin, flexible material that can be sealed to the upper surface of the disc using an adhesive or heat-bonded into place, such that it seals all fluid channels. It is also shaped such that fluid entry ports and air vents are not blocked.

Figure 4a illustrates the bottom surface of the reservoir layer **201**. Shown here

20 are a number of through features from Figure 2b, including the entry vias **204**. Also shown are the common reagent reservoirs **401** and **402**. Reservoir **401** distributes fluids along the lower face of the reservoir layer, while **402** distributes fluids through manifold **210** on the upper surface. Also shown are the overflow reservoirs **403** and **404** corresponding to reagent reservoirs **402** and **401**, respectively.

25 Figure 4b is a detail of the region around reagent reservoir **401**. The reservoir is accessed through hole **215** and entry passageway **406**; this may be shaped as shown to prevent flow of fluid toward the air vent **206**. In addition, the depth of the reservoir **407** can be contoured to prevent fluid from reaching the hole **206** before the remainder of the reservoir has been filled. The reservoir **401** is connected *via* a channel **408** to a

30 distribution manifold **409** through which the reagent is distributed. The fluid samples enter via ports **204** and channels **411**.

Figure 4c is a detail of the region around reagent reservoir **402**. The reservoir is accessed through hole **217** and entry passageway **412**; this may be shaped as shown to prevent flow of fluid toward the air vent **205**. In addition, a restriction in depth of the

reservoir **407** can successfully prevent fluid from reaching the hole **206** before the remainder of the reservoir has been filled. The reservoir **402** also contains a via **207** which communicates with the manifold **210** on the upper surface of the disc.

Figure 4d illustrates an expanded view of a section of the reservoir layer showing the reservoirs involved in a single assay. As shown in the Figure, this embodiment of the platforms of the invention contains three reservoirs plus one detection chamber for each assay. Each reservoir has dimensions of from about 0.05mm to about 5mm wide, from about 0.05mm to about 20mm long, and from about 0.05mm to about 5mm thick, and has a volumetric capacity of from about 0.1nL to about 500μL. Reservoirs **417**, **418**, and **416** are designed to contain fluids A$_i$(in some embodiments, this will the a sample), B, and C. For the purposes of this invention, reservoir such as reservoir **418** that is fluidly connected to the reagent manifold is terms an "aliquotted reagent reservoir". The detection cuvette for this assay is detection chamber **420** with air-port **213** leading to air displacement hole **214**. Air displacement holes **214** that allow air displaced by the motion of fluids to escape, have a cross-sectional dimension of from about 100 to about 500μm. These holes may optionally be replaced by a manifold or series of channels connecting the receiving reservoirs to one or more air holes. The detecting reservoirs are designed to be accessible to optical interrogation, *for example*, by being composed of optically-transparent plastics or other materials. Also shown is the distribution manifold **409** that communicates with the filling channel **415**. Filling channel **415** terminates at aliquotted reagent reservoir **418**. At the proximal portion of **415** a narrow passageway **414** is connected to filling channel **415**. Passageway **414** passes through one or more capillary junctions **413** to air port **212**. Reservoir **416** is connected via passageway **419** and via **211** to the distribution manifold **210** on the upper surface of the disc. Finally, reservoir **417** is connected *via* sample input port **204** to the interface

This collection of reservoirs and structures—**416**, **417**, **418**, **420**, **415**, **414**, **413**, **212**, **416**, **419**, **211**, **417**, and **411**—is repeated on the illustrated platform of the invention a total of 96 times azimuthally around the disc with an angular spacing of about 3.5°. The 96 arrays are sub-divided into two groups of 48; which are placed with azimuthal symmetry around the disc. Platforms having a smaller or greater number of arrays of such reservoirs are within the scope of the invention being most preferably

evenly spaced around the surface area of the platform in configurations that match the pattern of microfluidics components on the microfluidics layer.

Microfluidics Layer

The microfluidics layer of the embodiment of the platform of the invention is shown in Figures 5a through 5d.

Microfluidics layer **500** is optimally of the same lateral dimensions as the reservoir layer. There is also an optional center hole for mounting on a spindle, although this is not required in all configurations.

The microfluidics layer contains an array **501** of microfluidic structures **502**, the number of structures in the array being equal to the number of parallel assays to be run on the platform. In the embodiment illustrated in the Figures, there are 96 such structures evenly repeated with angular spacing of about 3.5°. Microfluidics structures **502** preferably comprise microchannels having cross-sectional dimensions of from about 5μm to about 500μm and a depth in the microfluidics layer of from about 10μm to about 3mm.

Figure 5b is an expanded view of a single unit of microfluidic structures. Each microfluidics structure comprises one microfluidics assay. The microfluidic structure consists of depressions in the surface of the microfluidic disc of a single or multiple depths ranging between 2 microns and 1000microns, while the widths of the depressions varies from about 2μm to about 500μm, as further described below.

The structure of the microfluidics components of the assay structure are as follows. Microchannels **505** and **506** are aligned by assembly between the reservoir layer and microfluidics layer so that the microchannels protrude into reservoirs **416** and **417**, respectively. Microchannels **505** and **506** in some embodiments narrow to form capillary junctions **508** before joining mixing microchannel **509**. Mixing microchannels are configured to provide mixing of different solutions as the mixture traverses the longitudinal extent of the microchannel. The degree of mixing is dependent on the flow rate of the fluids and the longitudinal extent of the mixing microchannel, which is proportional to the amount of time the two fluids are in contact and are mixed together. The degree of mixing is also dependent on the lateral extent of the mixing microchannel, and is further dependent on the diffusion constants of the fluids to be mixed. In order to accommodate mixing microchannels having sufficient

lengths for mixing fluids having a useful range of viscosities, the mixing microchannels are provided as shown in Figure 5b, wherein mixing is promoted as illustrated in Figure 5b by configuring the microchannel to bend several times as it traverses a path on the platform surface that is perpendicular to the direction of rotation, but extends radially on the surface of the platform from a position more proximal to a position more distal to the axis of rotation. Mixing microchannel **509** has a length of from about 1mm to about 100mm, its length in some cases achieved through the use of bends. Mixing microchannel **509** is provided with a capillary junction of a restriction in the lateral dimension at **510** wherein the interior diameter of the microchannel is reduced by about 0 to 95%, and then joins capillary junction **511**. Capillary junction **511** is larger in the lateral or vertical direction or both than the restriction **510**.

Mixing in the device is promoted through diffusion. If two small volumes A and B are added to a single container, diffusion of A into B and/or B into A will effect mixing. The amount of time required for this mixing will depend upon the diffusion constants of the molecules within the solutions whose mixing is desired and the distances over which the molecules must diffuse. For example, 0.5 microliter of solution A comprising a molecule with diffusion constant D is added to a reservoir 1mm on a side. Solution B comprising a molecule whose diffusion constant is also D is added. The solutions will initially occupy the volume with an interface partitioning them. Even if the fluids are highly miscible, the diffusion times to create a completely homogeneous solution will be approximately $t=2x^2/D$. For $x=0.05$cm (0.5mm) and $D=10^{-5}$cm^2/s, the mixing time is 500 seconds, an unacceptably long time for most reactions. This mixing time may be reduced by mechanical stirring, for example, but stirring is difficult to obtain in fluids confined in small structures because the flow of the fluid is laminar and does not contain turbulent eddies that are known to promote mixing. If, instead of placing fluids A and then B in a 1mm^3 container, fluids A and B were placed side-by-side in a long, thin capillary of lateral dimension d, the relevant time for mixing is much shorter. If, for example, d is 100 microns, mixing time t is 20 seconds. The mixing channels of the device simulate the placement of fluid in a long capillary by co-injecting fluid streams A and B into a capillary microchannel. These fluids flow side-by-side down the channel initially. As the fluid is pushed through the microchannel due to centrifugal force produced by rotation of the platform, diffusion

occurs between the fluids. By choosing a capillary of sufficiently narrow diameter, sufficient length, and a pumping rate that is sufficiently low, the portion of A and B of the total volumes of A and B present in the channel during pumping can be caused to mix.

These choices may be determined by setting the required time for mixing equal to the amount of time necessary for the fluid to traverse the channel. The required time for diffusion is

$$t_m \approx \frac{2w^2}{D}$$

where w is the lateral size of the channel. The amount of time necessary to traverse the channel is simply the length of the channel divided by the fluid velocity, the velocity being calculated as described in co-owned and co-pending U.S. Serial No. 08/910,726, filed August 12, 1997, and Duffy *et al.* (1999, *Anal. Chem.* <u>71</u>: 4669-4678):

$$t_t = \frac{l}{U} = \frac{l}{\left(\dfrac{\rho \omega^2 \Delta R \langle R \rangle (d^H)^2}{32 \eta l} \right)} = \frac{32 \eta l^2}{\rho \omega^2 \Delta R \langle R \rangle (d^H)^2}$$

where the fluid properties are the density ρ and viscosity η, ΔR and $<R>$ are the extent along the radius and average radial position of the fluid subject to centripetal acceleration, and l and d^H are the length and hydraulic diameter of the channel. By choosing variables such that t_t is at least equal to or greater than t_m, mixing in the microchannels is achieved.

Entry 512 to microchannel 514 protrudes into aliquotted reagent reservoir 418 and preferably forms capillary junction 513, having dimensions substantially the same as capillary junction 511. Microchannel 514 passes through a restriction in the lateral dimension at 515 wherein the interior diameter of the microchannel is reduced by about 1-99%, and then joins capillary junction 511. The capillary junction leads to a further mixing microchannel 516 that terminates at end 517 and that protrudes into detection chamber 420. Mixing microchannel 516 has a length of from about 1 mm to about 100mm.

Additional structures on the microfluidics disc include overflow reservoirs 503 and 504 as shown in Figure 5c and 5d. Each overflow structure abuts at 518 with the terminus of a distribution manifold. Entry 518 passes through passageway 519 to

enlargement **520**, forming a capillary junction. This is then connected *via* channel **521** that ends at **522**, internal to an overflow chamber.

Structure of the Assembled Microsystems Platform

5   Figure 6 illustrates three assay sectors of the assembled platform, in which the reservoirs of the reservoir layer are mated to microchannels from the microfluidics layer. The platform layers are mated as described in more detail below.

         Because the. principles by which the fluidic elements of the platforms are combined are understood, these platforms can be used for a variety of bioanalytical

10   methods. Passive or capillary valving of two fluids to bring them into a channel and the use of that channel to facilitate fluid mixing by diffusion may be used to include any number of fluids, and is not limited to the mixture of two fluids followed by further combination of the first mixture with a third fluid, as illustrated herein. In addition, since the mixing ratios depend on the geometric shapes of the reservoirs containing the

15   solutions to be mixed (as described more fully in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000 and incorporated by reference herein), alternative arrangements of these geometries result in mixing ratios over a large range.

         Similarly, capillary valving is understood to depend on geometry, fluid properties and rotational rate, as disclosed more fully in U.S. Serial Nos. 08/761,063,

20   filed December 5, 1996; 08/768,990, filed December 18, 1996; and 08/910,726, filed August 12, 1997, incorporated by reference herein. Alternative arrangements of the microfluidic layers of the platforms of the invention can be provided to contain any number of concentric rings of assays consistent with the amount of surface area available on the platform surface and the extent of the surface taken up by any one

25   embodiment of microfluidics required to perform an assay.

         The fluid channels described here are preferably of a size that the residence time within the channel of a fluid element under centrifugal flow is sufficient to allow diffusional mixing across the diameter of the channel. The design of such mixing elements is defined in co-owned and co-pending application U.S. Serial N0.

30   09/595,239, filed June 16, 2000, incorporated by reference.

         An example of alternative platforms for performing assays of the general form disclosed herein, having a number of reservoirs whose volume is equal to the total fluid volume of an assay, VA, connected by microchannels to a collection/detection chamber having the same volume VA. For reservoirs having a common depth, *t*, and

microchannels and inter-assay areas occupying approximately the same area as the reagent reservoirs and collection/detection chambers, the total number of assays possible on a disc of radius R is approximately

$$\text{Number of assays} = \pi R^2 t/(4VA)$$

For a platform having a radius of R=6cm, a reservoir depth of 0.1cm and a total fluid volume of 4 microliters, the total number of assays that can be fit onto the disc is 706.

Other considerations include the placement of the components on the platform relative to the axis of rotation. Generally, the collection/detection chamber should be closer to the edge of the platform than the reagent reservoirs, so that there will be sufficient hydrodynamic pressure produced by convenient rotational speeds to motivate the fluid through the microchannels and mixing elements and into the collection/detection chambers. Placement of the collection/detection chambers at the outer edge of the platform also facilitates detection using a fixed optical detector. However, for extremely high-density platforms this may not be the most efficient way to arrange the assay components. For example, if the desired number of assays can be achieved only by placing the collection/detection chambers nearer the reservoirs containing the unreacted samples and reagents, it may be necessary to use a detector that can access cuvettes at a variety of radial and azimuthal positions. An example of a scanning optical system would be one in which the optical signal is scanned radially, while the disc could be indexed beneath the optics azimuthally. In this way the optics can address any point on the disc surface. Scanning methods include a detector on a linear drive that moves radially; alternately, the optical signal may be scanned radially through the use of a galvanometrically-controlled mirror system.

The platform of the invention is used for the performance of assays in the following fashion, referring to Figures 7 and 8, which illustrate fluid motion in the vicinity of the reservoirs **401, 402, 403, 404** and an assay structure, respectively. The assays were run as follows. Aliquots of fluid A are added ports **203+204**; the volume of the individual aliquots are identical, but may range depending upon the design of the device from 1nL to 50 microliters. The chosen volume for the aliquot is such that, when fluid fills reservoir **417**, it protrudes into **411** for a short distance, typically less than 1/3 of the length of **417**. A total volume of fluid B equal to the number assays

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multiplied by the volume of fluid B required for each assay, plus an additional volume greater than or equal to the volume enclosed in channels **409** and in all channels **415**, is added reservoir **401** through entry port **215**. Figures 7 and 8 illustrate the sequence of fluid flows in the vicinity of the reservoirs of microfluidic assay elements. Figure 7a illustrates the flow of fluids entering reservoirs **401** and **402**, as well as of samples of fluid A enter ports **217** and channels **414**. The platform is then placed on a device that is capable of performing rotations. At a first rotational speed ranging from 100-1000 rpm, fluids A are driven through channels **411** into the reservoirs **417**, where they are retained by capillary junctions**508**. This is illustrated in figures 7a-7d and 8a through 8d. Also at this rotation rate, fluid B is driven through hole **207** into manifold **210**. As fluid B travels **210**, it flows through the vias **211** into passageways **419** and finally reservoirs **416**, where it was retained by capillary junctions **508**. Fluid B also pases through via **218** and is retained at capillary junction **520** of overflow structure **503**. Also at this rotation rate, fluid C is driven through channel **408** and manifold **409** to the fill channels **414**, and then to aliquotted reagent reservoirs **418**. Fluid in aliquotted reagent reservoir **418** is stopped at capillary junction **513**. Fluid C also enters capillary **414** and is stopped at capillary junction **413**. Fluid C completely fills **409** and is also stopped at capillary junction **520** over overflow structure **503**. The rotational rate is then increased to a second speed. At this speed, the overflow capillary valves formed **520** on the overflow structures **503** and **504** release. Fluid A in manifold **210** flows into the overflow reservoir **403**, leaving behind solution in the reservoirs **416**. Similarly, fluid C flows into reservoir **402**; as it flows, the fluid in each assay structure at capillary junctions **413** is "pulled". However, the fact that aliquotted reagent reservoir **418** is radially-outward from **413** means that there is rotationally-induced resistance to drawing the fluid from aliquotted reagent reservoir **418**. The tension in the fluid at **413** is relieved through the introduction of air via the channel and port **212**; by introduction of this air bubble, solution C within the manifold **409+415** is effectively separated from fluid remaining in aliquotted reagent reservoir **418**. The speed is then increased to a third value, at which point the capillary junctions at **508** and **513** allows fluid to flow. The fluids from reservoirs **505** and **506** flow through channel **509** and are halted at the junction **510**; similarly, the fluid flowing through **514** was halted at the junction **515**. At a fourth rotational rate, the capillary junctions at **511** allow the fluids to flow. The mixing fluids are then pumped via centrifugation into the detection reservoir **420**. In the case of the junctions **507** and **511**, whichever fluid flows first is forced to wet the

exit capillary of the other fluid in the capillary junction, thereby inducing it to flow as well.

In some alternative constructions, the various rotational rates need not be monotonically increasing. The velocity may be "spiked" momentarily from a low value to high value when a capillary valving event is desired; if it is then reduced quickly to a lower value, the next capillary valving event may be designed to operate at the same rotational rate as the first, or even a lower rate. By using the delay time required for fluid to transit from capillary valve to capillary valve in a sequence, a large number of events may be designed to function serially.

Three alternative constructions of the fluidic design are now discussed. Figure 9 illustrates a detail of the lower face of the fluidics layer 201 of the platform depicted in Figures 1 through 5. Most of the features illustrated correspond to those of Figures 2 through 4. It will be understood that the structures in microfluidic layer 500 are sized and spaced so that they mate with those of layer 201 in the preceding example. The additional elements in this embodiment are capillary 901, a combined capillary junction and overflow chamber 902, and air-vent 903. This embodiment is designed to meter an imprecise volume of "sample" fluid for each assay structure. The function of this alternative is the same as in the previous embodiment, with these additional features: At the first rotational speed, sample fluid is delivered *via* channel 411 to reservoir 417. As it flows to the reservoir, some fluid enters capillary 901 but is retained at the expansion of 901 into capillary junction 902. The fluid fills the reservoir and is halted by capillary junction 508 in the microfluidics layer (not shown in this figure). The dimensions of capillary 901 are chosen so that fluid is able to pass opening 902 at a rotational speed intermediate between the first and second rotational speeds discussed above. Displaced air is vented through 903, and fluid which extends radially-inward of the inersection of cahnnel 411 and capillary junction 902 flows into capillary junction 902. Air vent 903 may be chosen to be small enough that fluids cannot escape it at any rotational speed normally used for the device. The selection of the diameter of capillary 901 depends on the expected amount of excess volume. For example, for an assay volume of 0.5µL and a conventional pipetting device used to apply fluids to the disc having a precision of ±0.2µL. The device may be designed so that the user is required to add 0.75µL, as long as the volume of channel 901 is 0.05µL or less. For example, if the radial position of 902 is the same as that of capillary junction 508 on microfluidic

layer **500**, the diameter of capillary **901** at capillary junction **902** need only be somewhat larger than that of the channels that meet at capillary junction **507** in order to function. A 100μm wide and 100μm deep channel **901** meets requirements.

A second alternative construct is shown in Figure 10. In this embodiment, the manifold **210** is positioned radially-outward of reservoirs **416, 417,** and **418**. Via **211** connects with channel **1003** that leads radially-inward to a microchannel configuration identical to that associated with aliquotted reagent reservoir **418** described above, consisting of capillary **1004**, capillary junctions **1005**, and air vent **1006**. It will be understood that this structure behaves like that associated with aliquotted reagent reservoir **418** in the previous description, by isolating fluids in the multiple reservoirs **416** from one another. Modifications in which channel **1003** resides on the "upper" face of the layer **201** are within the scope of the invention, as long as a via connecting the fluid path to the lower face is made, for example, at the radially-proximal end of **416**; this may be advantageous in order to "pack" structures most effectively in the azimuthal direction.

This alternate construction may provide advantages for liquids that have unpredictable properties such as viscosity and surface tension. With such fluids, it is possible that bubbles may accidentally be introduced in manifold **210** in the previously described embodiment. These bubbles make draining of the manifold, and isolation of the reservoirs **416**, difficult. By introducing bubbles at each reservoir, the need for maintaining a single plug of fluid in manifold **210** is relaxed

A third alternative construction with additional functionality is shown Figure 11. In this Figure, a microfluidic network for the creation of a dilution series is illustrated and is part of a larger network of structures used for various purposes. The fluid distribution scheme illustrated here is advantageous not only for the three-fluid homogeneous assays disclosed herein but can be applied to any centrifugally-based process which requires the creation of such dilution series.

In this embodiment, the platform has only three ports and reservoirs for fluid addition: Reservoirs **401** and **402** for common reagents, as previously discussed, and reservoirs **601** and **602** placed on a surface of the platform **201** and accessed by entry ports **618** and **619** respectively. Reservoirs **401** and **402** lead to distribution manifolds, overflows, and assay reservoirs as previously discussed. Fluid channel **603** exits reservoir **601** and is split into two components at what will be called a T-junction **604**, a

portion of which continues to further T-junctions and a portion of which, **607**, terminates at a capillary junction **609**. Similarly, reservoir **602** leads to channel **605**, which is split at T-junction **606**; one arm of the split channel continues to further T-junctions, while the other arm, **608**, terminates at the capillary junction **609**. Following channel **603** past T-junction **604**, it is again split at T-junction **610** into a portion which leads to reservoir **417** of the left-most assay structure **650**. The structure **650**, composed of reservoirs and channels as previously disclosed, embodies the same functionality as that given in Figure 6. Channel **419**, for example, is the passage for the entry of first reagent into reservoir **416**. The other portion of channel **603** terminates at capillary junction **615**. Similarly, channel **605** leads to T-junction **612**, where it is split into channel **613** which terminates at capillary junction **618** and a portion which continues to assay structure **651**. The capillary junctions **609**, **615**, and **616** all are fluidly connected to channels **614**, **640**, and **641**, respectively. Channels **640** and **641** lead respectively to assay structures **653** and **654**. Channel **614** is further split at a 4-armed junction **617** into 3 channels: A continuation of **614**, which leads assay structure **652**, and side channels **618** and **619** which terminate at capillary junctions **615** and **616**, respectively.

The disc may be used to distribute liquids for an assay in the following fashion. Common reagents are loaded into reservoirs **401** and **402**. "Sample" (fluid A) is loaded into reservoirs **601** and a diluent buffer (fluid B) into reservoir **602**. Under the influence of rotation, the common reagents are distributed to reservoirs **416** and **418** as previously described. Sample and diluent enter channels **603** and **605**. Fluid A reaches the T-junction **604**, at which point a portion of the fluid continues down channel **603** and a portion flows into channel **607**. Similarly, fluid B splits at **606** into channels **605** and **608**. The portion of A present in **607** reaches capillary junction **609**, as does the fluid B present in 608. As the disc is spun to overcome capillary force at **609**, the fluids are brought together and flow into meandering mixing channel **610**. Mixing in this channel is described in co-owned and co-pending application U.S. Serial N0. 09/595,239, filed June 16, 2000, incorporated by reference. The fluid in channel **614**, after sufficient time for diffusional mixing in the channel, arrives at junction **617** with a volume fraction of A equal to 0.5 and B equal to 0.5, *i.e.*, the fluids A and B are "mixed". The mixed fluid arriving at **617** is denoted fluid C1.

Fluid C1 is split into 3 streams at junction **617**. A portion of that mixed liquid C1 now mixes with the original A solution which has been directed by channel **603** to junction **610** and channel **611**, by passing through capillary junction **615**. This fluid,

denoted C2, is in channel **640** and has volume fraction of A of 0.75 and B of 0.25. Similarly, the fluid in **641** has volume fraction of A of 0.25 and B of 0.75.

The functioning of the remainder of the network of channels is clear from this demonstration. As shown, the fluidic network delivers 5 concentrations of A—1.0, 0.75, 0.5, 0.25, 0.0—to the structures **650, 651, 652, 653,** and **654,** respectively. In order to achieve these ratios, the flow rates of the two fluids entering any mixing channel **614, 640,** and **641** must be equal. This is assured by the diameter of the channels, as fluid flow is controlled by the fluidic impedances of the various mixing channels.

It will be understood that the process of dividing and recombining channels illustrated may be continued indefinitely. One further splitting and recombination in the manner shown would lead to a total of 9 concentrations of A: 1.0, 0.875, 0.75, 0.625, 0.5, 0.375, 0.125, 0.0625, 0.0.

It will further be recognized that this mixing scheme need not be restricted to use with the three-fluid homogeneous assays previously discussed, but may be used to deliver a fluid of arbitrary composition to a point on the platform.

A number of variations in fluidic design are possible, either dictated by assay requirements, fluidic requirements, ease-of-use or reduction in automation or all of these factors. For example, capillary valves have been shown to retain fluids in an intermediate chamber at elevated temperatures, used for incubation (as disclosed more extensively in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000, incorporated by reference). Assays that require intermediate incubations, *for example*, because of slow chemical kinetics, may be performed in such structures.

Alternatively, assays for which diffusional mixing is insufficient may require agitation of the fluid to effect mixing. In such a case, active valves can be used, which retain the fluids against the sudden pressure changes induced by agitation, as described in more fully in co-owned and co-pending U.S. Serial No. 09/315,114, filed May 19, 1999.

It may also be desirable to treat the platform surfaces to change the liquid contact angle for controlling capillary valving properties, as disclosed in co-owned and co-pending U.S. Serial No. 08/910,726, filed August 12, 1997, incorporated by reference.

The following Examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature.

EXAMPLE 1
Simultaneous Enzyme Inhibition Assays

A platform depicted in the Figure 11 was used in this example. The platform of Figure 11 is functionally identical to that of Figures 1-8, the only significant difference being that that of Figure 11 is designed for the performance of 24 assays, while that of Figures 1-8 is designed for the performance of 96 assays.

The platform was prepared as follows. The macrofluidic reservoir layer **201** was manufactured through machining of acrylic using computer/numerical code machining using a Light Machines VMC5000 milling machine running Light Machines "Benchman" software (Light Machines Corporation, Manchester, NH).

The sealing film of as shown in Figure 3 was made by applying a double-sided tape to a thin sheet of heat-stabilized polyester (mylar). A section of mylar bonded to tape was cut from the combined sheet to the correct shape, leaving one adhesive face of the tape for application to the macrofluidic layer.

The microfluidics layer was manufactured as follows. A microfluidics structure such as the structure shown in Figures 5a through 5d was designed using in a computer aided design package such as AutoCAD (Autodesk, San Rafael CA) and Freehand (Macromedia Inc., San Francisco, CA). This design was converted into a photomask by printing at high resolution (3386 dpi) on a transparent plastic sheet. A 125-mm diameter silicon wafer was coated with a layer of negative photoresist (SU-8(50)) and spun on a spin-coater (Chemat Technology, Northridge, CA) at a speed sufficient (200 to 8000 rpm) to give a desired thickness between 5μm and 500μm. The silicon wafer was baked to have a smooth surface and then the photoresist partially cured. The silicon wafer was exposed to ultraviolet (UV) light using a conventional UV source and mask aligner. The photoresist was then developed in propylene glycol methyl ether acetate and non-crosslinked photoresist removed through washing in dichloromethane. The resulting relief was then passivated by exposure to a vapor of tridecafluoro-1,1,2,2-tetrhydrooctyl-1-trichlorosilane and used as a mold for microfabrication (Duffy *et al.*, 1998, *Anal. Chem.* 70: 4974-4984).

A 10:1 mixture of polydimethylsiloxane (PDMS) oligomer and crosslinking agent (Sylgard 184, Dow Corning) was poured onto the mold after degassing under vacuum. PDMS is a clear material; by adding 1 wt% liquid pigment (containing TiO_2),

the disc was made white for reflectance optical measurements and black for fluorescence measurements. The poured elastomer was then cured at 65°C for 1 hour. The resulting microfabricated PDMS part was peeled from the mold. The mold could then be re-used to fabricate additional copies of the microfluidics layer.

The microplatform was assembled in the following way. Sealing film with adhesive exposed was first applied to the upper surface of the macrofluidic layer so that it completely covered the holes and channels **207, 208, 211, 218**. Final assembly was completed by forming a reversible, conforming seal between the PDMS microfluidics layer and the bottom surface of the macrofluidic reservoir layer **201** made through simple physical contact of the two components. This seal is based on physical adhesion forces alone—van der Waals attraction forces and potentially static electrical charge present on the surfaces—and was sufficient to seal the disc against leakage due to the centripetally-induced pressures used.

The platform shown in Figure 11 and prepared as described herein was used to perform simultaneously and in parallel 24 enzyme inhibition assays. Fluids were deposited in the reservoirs formed in reservoir layer **201** when reservoir layer is mated or bonded with microfluidics layer **500**. Platform **100** is then rotated using a rotational profile designed to drive fluids through the channels within the macrofluidic disc **201** and the microfluidic disc **500**.

The platform shown in Figure 11 possesses the same features as that shown in Figures 1 through 6, and reference to features will be made those on the latter figures. It was used to perform 24 simultaneous enzyme inhibition assays as model homogeneous assays. In an enzyme inhibition assay, the effect of a compound present in a first fluid ("A") upon the capacity of an enzyme present in a second fluid (fluid "B") to catalyze a reaction, typically of a substrate in a third fluid ("C") is determined. The reaction was chosen to give a change in a readily-measured parameter of the fluid, such as its optical density, or to produce a fluorescent moiety. When no inhibitor was present in fluid A, mixing solution A with enzyme solution B had no effect: The enzyme activity detected in this assay was the maximum detected and provided the largest change in the measured parameter. However, if an inhibitor was present in fluid A, mixing fluid A with fluid B will, after a sufficient time resulted in a chemical reaction or other change induced by the inhibitor in most or all of the enzyme molecules, rendering them

incapable of catalyzing the desired reaction. If this solution was mixed with the substrate solution, little or no change in the measured parameter was seen.

The system chosen to model homogeneous assays consisted of theophylline as inhibitor, alkaline phosphatase as the enzyme, and p-nitrophenol phosphate (PNPP) as the substrate. In the presence of alkaline phosphatase, PNPP, which is colorless, is converted to p-nitrophenol (PNP), which absorbs in the blue and therefore appears yellow. Theophylline was used in concentrations of 0.01mM to 100mM to provide a standard dose-response curve in the inhibitor. Alkaline phosphatase was used in a 1mg/mL solution. PNPP was used as a 0.5mM solution. All solutions were made in a buffer of 0.1M glycine and 0.5mM $MgCl_2$ in deionized water.

The dimensions of the platform used for these assays were as follows. The overall platform diameter was 12cm. The macrofluidic disc was about 1.6mm thick. The diameter of the sample entry ports through-holes 204 was 0.5mm; the depression 203 was conical, with an outer diameter of 2mm and a depth of 1mm, allowing the cones to "guide" the placement of pipette tips when loaded manually. The common reagent entry ports consisted here of only through holes 215 and 217, also of diameter 0.5mm. The air ports 205 and 206 were also 0.5mm in diameter. The reagent manifold through-hole 207 was also 0.5mm in diameter, and the exit channel 208 was of larger diameter and depth of 0.43mm. The exit channel narrowed to join the channel 209 of width 0.45mm. The manifold 210 was also 0.43mm deep and0.45mm wide. The vias 211 were 0.45mm in diameter and penetrated through the macrofluidic disc from the top surface to the bottom surface. The terminal via 218 had a diameter of 0.53mm and penetrated to the bottom surface of the layer.

The dimensions of the features on the lower face of the macrofluidic disc were as follows. The common reagent reservoirs 401 and 402 had a depth of 1.14mm. The radial positions of the ends of these reservoirs most proximal to the center of the disc were 1.59cm and that of the ends most distal from the center of the disc were 3.4cm. The volume allowable in reagent reservoir 402 was 60μL, while that of reservoir 401 was 120μL. Entry passageway 406 was 0.25mm deep and 0.25mm wide. Channel 408 was 0.25mm wide x 0.25mm deep, as was manifold 409. Overflow reservoir 403, corresponding to reagent reservoir 402, had an outer radius of about 5.6cm and an inner radius of about 4.3cm and a depth of 1.5mm. The angle subtended by the reservoir was chosen to accommodate a volume of 30 microliters. Similarly, overflow reservoir 404,

corresponding to reagent reservoir **401**, had an outer radius of about 5.6cm and an inner radius of about 4.3cm and a depth of 1.5 mm. The angle subtended by the reservoir was chosen such that it could accommodate a volume of 30 microliters. Air holes **410** had a diameter of 0.5mm.

Referring to Figure 4d, the features of the individual assay structures were as follows. Entry channels **411** were 0.25mm wide x 0.25mm deep; their lengths varied according to the relative positions of the reservoir **417** and entry port **204** that each channel connected, from about 12mm to about 27mm. Channel **411** preferably opens at its connection to reservoir **417** such that acute angles are not presented within the plane of the disc that might impede fluid flow. The inner radii of reservoirs **416, 417,** and **418** were about 4.1cm and outer of reservoirs **416, 417,** and **418** were about 4.5cm. Both inner and outer ends of the reservoirs were rounded with radii of 0.5m to prevent fluid from both "stopping" at the inner end during loading due to capillary forces and being retained as the reservoirs emptied. Reservoirs **416, 417,** and **418** were about 0.57mm, 0.57mm, and 1.14mm deep, respectively. Each reservoir was 0.6mm in width. The lengths, depths, and widths of the reservoirs were chosen such that the volumes contained within **416** and **417** were 0.91microliters while that contained with aliquotted reagent reservoir **418** was twice that volume, 1.82 microliters. Detection reservoirs **420** were constructed of optically-transparent material and had an outer radius of about 5.7cm and an inner radius of about 5.2cm, were 0.7mm deep, and had a subtended angle of 3.2° and were thus designed to accommodate the combined volumes of reservoirs **416, 417,** and **418** (approximately 3.6 microliters). Distribution manifold **409** is connected to aliquotted reagent reservoir **418** *via* a filling channel **415** which as 0.25mm wide x 0.25mm deep; the end of **415** was also enlarged at its connection to aliquotted reagent reservoir **418**. Capillary channel **414** was 0.13mm wide x 0.13mm deep. The capillary junctions **413** were 0.25mm deep x 0.5mm wide; they were formed so that the opening of **414** into **414** formed a backward angle of 45°, thus increasing the capillary stopping power of the junction. The connection to reservoir **416** is *via* passsageway **419**, which was about 0.25mm deep and widened by 0.25mm wide to 0.6mm wide at its joining with **416**.

The microfluidics layer **500** was also 12cm in diameter and had a thickness between 1 and 2mm (although the thickness is not important) and was composed of white PDMS. The depth of all microfluidic structures (that was determined by the height of the SU-8 relief) was 100μm. The width of the entrance to the mixing

channels, **505, 506,** and **512,** was 200μm. The channels narrowed to 100μm before reaching the capillary junction at **508.** The width of the junction **508** was 120μm. Similarly, the entrance **512** narrowed to 100μm prior to the junction **513** that was 200μm. The widths of channels **509** and **514** was 100μm. The lengths of the mixing channels was chosen to provide sufficient time for mixing *via* diffusion with liquids of moderate diffusion constant ($5 \times 10^{-6} cm^2/s$) as fluids are pumped through them under the influence of centripetal acceleration. The lengths of channels **509** and **514** was about 17mm. These channels narrowed to 50μm at **510** and **515** before joining the junction **511,** which was about 0.4mm square. Channel **516** was 100μm wide and about 38mm long. These dimensions resulted in the fluids taking $\geq$ 2sec to transit the mixing microchannels.

Also on the microfluidic layer are the overflow structures **503** and **504.** The entry **518** was 0.4mm x 1.8mm, while the capillary passageway **519** was 120μm wide. Enlargement **520** was about 200μm wide by 200μm long, and **521** was also 100μm long.

The assays were run as follows. 1μL aliquots of theophylline solutions having the concentrations set forth above were loaded into entry ports **203+204** using a pipette. A total of 40μL of alkaline phosphatase was loaded into reservoir **402** through entry port **217,** and a total of 10μL of PNPP solution was loaded into reservoir **401** through entry port **215.** The platform was placed on the spindle of an instrument containing a diffuse reflectance optical head capable of three-color measurements. Figures 7 and 8 illustrate the sequence of fluid flows in the vicinity of the reservoirs of microfluidic assay elements. The platform was first rotated at 300rpm for 30seconds. At this rotation rate, the theophylline solutions were driven completely through channels **411** into the reservoirs **417,** where it was retained by capillary junctions **508.** Also at this rotation rate, the alkaline phosphatase solutions were driven through hole **207** into manifold **210.** As the alkaline phosphatase solution traveled **210,** it flowed through the vias **211** into passageways **419** and finally reservoirs **416,** where it was retained by capillary junctions **508.** Also at this rotation rate, the PNPP solution was driven through channel **408** and manifold **409** to the fill channels **415.** The PNPP solution was driven through **414** to the aliquotted reagent reservoirs **418;** PNPP solution also entered capillary **414** and was stopped at capillary junction **413** and at capillary junction **513.** The rotational rate was then increased to 500rpm. At this speed, the overflow capillary

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valves formed by **519** and **520** on the overflow structures released. The alkaline phosphatase solution in manifold **210** then flowed into the overflow reservoir **403**, leaving behind solution in the reservoirs **416**. At the same time, the draining of excess PNPP solution into the overflow reservoir **402** exerted an inward "pull" on the solution at the capillary junctions **413**. The tension in the fluid in channel **415** so created was relieved through the introduction of air via channel **413**; this effectively separated the draining PNPP solution in **415+409** from the solution in the aliquotted reagent reservoirs **418**. The speed was increased to 600rpm, at which point the capillary junctions at **508** and **513** allowed fluid to flow. The fluids from reservoirs **505** and **506** flow through channel **509** and were halted at the junction **510**; similarly, the fluid flowing through **514** was halted at the junction **515**. At 700 RPM, the capillary junctions at **511** allowed the fluids to flow. The mixed fluids were then pumped into the detection reservoir **420**. In the case of the junctions **507** and **511**, whichever fluid flows first is forced to wet the exit capillary of the other fluid in the capillary junction, thereby inducing it to flow as well.

An important feature of mixing in the device is made possible through the narrowness of microchannels **509**, **514**, and **516**. The resistance to flow due to rotationally-induced pressure of a channel that is denoted by R_H is given by

$$Q = \frac{P}{R_H}$$

$$R_H = C \frac{l}{\left(d^H\right)^4}$$

where Q is a flow-rate, P is the induced pressure, C is a constant, l is the length of the channel through which the fluid flows and d^H is the hydraulic diameter. Because the diameter of microchannels **509**, **514**, and **516** are much narrower than that of the reservoirs **416**, **417**, and **418**, resistance to flow is dominated by the microchannels, and hence the pressure drop across the flowing fluid is sustained almost exclusively over the length of the mixing microchannels. This insures that the fluids flowing from reagent reservoirs into mixing channels do so in a strict, known ratio. In particular, assume that fluid begins to flow from one reservoir into the mixing channel at a rate faster than the fluid flows from the other reservoir. The resulting pressure drop from the meniscus of the fluid at the inner edge of the reservoir to the point where the fluids mix for the fluid that flowed faster will be less than that of the other fluid, because the rotationally-induced pressure is proportional to the radial extent of the fluid (ΔR discussed earlier).

Because a higher pressure now exists across the fluid that moved more slowly, it is induced to flow more rapidly. This process of feedback provides a pressure-equalization phenomenon that results in the inner meniscuses of fluids in reservoirs **416**, **417**, and **418** progressing outward at the same radial velocity (same distance in the radial direction per unit time). As a result, the ratio of the alkaline phosphatase and theophylline flow-rates as a function of time in mixing microchannel **509** is given exactly by

$$\frac{Q_A}{Q_B}(t) = \frac{A_A}{B_B}(t)$$

where A_A and B_B are the cross-sectional area of the reservoirs **416** and **417** as a function of time, or alternately, radial position of the meniscus as fluid is removed from the reservoirs. If it was desired that the ratio of flows is constant (as was the case here), it was sufficient to maintain a constant ratio of cross-sectional areas as a function of radial position. Note that this does not imply that the cross sections are constant, just that their ratio is. The ratio expressed in the equation can be manipulated by altering the ratio of cross-sectional areas of the reservoirs, as disclosed more fully in co-owned U.S. Patent No. 6,063,589, issued May 16, 2000 and incorporated by reference.

This equation and analysis also accurately describes the significance of the ratio of the three fluids in mixing microchannel **516**. Because microchannel **509** and **516** are long and the flow rates can be controlled by rotational rate, the co-flowing streams are present in those channels for a time sufficient enough for diffusion across the interface between these streams to effect complete mixing of the solutions.

After fluid was delivered to the detection reservoirs **420**, reflectance optics was used to measure the reflected radiation at an off-specular (diffuse) angle at two wavelengths, 430nm (absorbing for the expected reaction product, PNP) and 630nm. As there is no absorbance from reaction product PNP at 630nm, this wavelength can be used to correct for optical imperfections in the platform, stray scattering, or unintended air bubbles in the optically-transparent chamber. The optical system also advantageously contained a beam-splitter that sent a fraction of the incident light to a reference photodiode. Two detectors used in this optics system were the assay detector, which measured diffusely-reflected light; and the reference detector, which measured a fraction of the incident light. Measurements at each detector were made when both the 430nm and 630nm light sources were active as well as when they were "dark" or off. The measured voltages were thus:

$V_D{}^D$ dark measurement in assay detector

$V_R{}^D$ dark measurement in reference detector

$V_D{}^1$ measurement at absorbing wavelength λ_1 (430nm) in assay detector

$V_D{}^2$ measurement at non-absorbing wavelength in λ_2 (660nm) assay detector

$V_R{}^1$ measurement in reference detector at absorbing wavelength λ_1 (430nm)

$V_R{}^2$ measurement in reference detector at non-absorbing wavelength λ_2 (660nm)

The absorbance at 430nm is calculated from

$$K = \frac{\left(\dfrac{V_D^1 - V_D^D}{V_R^1 - V_R^D}\right)}{\left(\dfrac{V_D^2 - V_D^D}{V_R^2 - V_R^D}\right)}$$

$$A = -\log(K) \propto c_{PNP}$$

Here, C_{PNP} is the concentration of yellow product, *p*-nitrophenol; this concentration is inversely related to the concentration of theophylline in the initial solution.

Data was collected continuously as the platform was rotated at 60 or 100 rpm. Because data can be taken continuously, the kinetics of the chemical reactions could be observed. Figure 12 shows data for 24 assays run simultaneously on the platform, representing four-fold replicates for each of six theophylline concentrations ranging from 0 to 10mM. The data are consistent with those generated on the laboratory bench using absorbance and that contained in co-owned and co-pending U.S. application Serial No. 09/595,239, filed June 16, 2000, incorporated by reference herein.

These results demonstrated that microplatform systems according to the invention can be used as a substitute for conventional 96-well microtiter plates for performing enzyme assays to determine enzymatic activity thereof.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention.